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Proceeding Paper

Development of Breads Fortified in Calcium and High Protein Content through the Use of Bean Flour and Regional Fruits †

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Abstract: The World Health Organization recommends the consumption of legumes and considers them good allies to achieve food security and reduce malnutrition worldwide. Bread offers the possibility of incorporating ingredients to improve one's diet without changing their eating habits. The objective of this study was to formulate and elaborate calcium-fortified bread, optimizing nutritional quality using protein supplementation with regionally produced ingredients, which are underutilized for domestic consumption. The variability of the protein quality of wheat flour and its mixtures with bean flour was studied. The proteins, fats, dietary fibre, ashes, and moisture were determined using AOAC methods. Volume, texture, and colour were also evaluated using a Vernier caliper, a TAXT plus Texturometer, and a Colour Quest XE spectrophotometer, respectively. The addition of calcium salts increased hardness, produced lighter crumbs and crust, and did not affect volume. The addition of fruit pulp did modify the colour and volume of the loaves. The moisture, protein, calcium, and sodium content of the baked goods were 42 g, 11.6 g, 443 mg, and 347 mg per 100 g of bread, respectively. A sodium reduction of 30% was obtained with the consequent increase in calcium, both critical nutrients by default. The breads produced are inexpensive and have higher contents of high-quality protein and calcium. Due to their ingredients and their nutritional and textural characteristics, the breads could be incorporated into the diets of vulnerable groups and contribute to the prevention of chronic and/or deficiency diseases. In addition, the use of regional products will encourage local production and therefore support the local economy.

Keywords: added calcium; breads; legumes; fruits; regional

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1. Introduction

The world population has grown steadily, and most people now live in urban areas. Technology has evolved at a dizzying pace, while the economy has become increasingly interconnected and globalized. Climate change with the increasing variability of climate and extreme events is affecting agricultural productivity, food production, and natural resources with impacts on food systems and means of rural livelihoods, including a decrease in the number of farmers. All of this has led to major changes in the ways in which food is produced, distributed, and consumed worldwide, and to new food security, nutrition, and health challenges [1] Globally, most markets offer a wide variety of foods and beverages that combine flavour, comfort, and novelty. However, at the same time, there is a wide availability and widespread marketing of many of these products, especially those with a high content in fat, simple sugars, and/or salt. These dietary patterns have an impact on the nutritional status of the Argentine population in general and the NOA population in particular, which has high rates of overweight and obesity that coexist with high rates of malnutrition and low weight, as well as some micronutrient deficits [2,3]. Adding to the epidemic of overweight and obesity, which is the most frequent form of malnutrition and continues to increase steadily in Argentina, is the COVID-19 pandemic [4].

On the one hand, legume flours provide protein, fibre, and micronutrients while reducing fat consumption. They are the fundamental protein and caloric base of human nutrition and the main source of energy and essential amino acids provided in a balanced and nutritious way, especially when they are combined with cereals. Populations in the interior of the country have higher consumptions of dried legumes, compared to the Federal Capital and Greater Buenos Aires. However, in previous studies carried out in the NOA population, little or no consumption of these was observed [2]. The market for legume flour is mainly driven by the health benefits that it provides. When cereal proteins and legumes are combined, the biological value of each of them is improved, making an opportunity to create new foods of high nutritional and organoleptic quality. On the other hand, pitanga (*Eugenia uniflora* L.) is an edible fruit and botanical berry. These native fruits can be an important source of antioxidant and nutraceutical compounds due to the content of calcium, phosphorus, anthocyanins, flavonoids, carotenoids, and vitamin C comparable or superior to others. They are generally consumed fresh, but due to their highly perishable nature, they are also processed into a range of value-added industrialized products such as juices and fruit syrups, among other products [5].

The objective of the present study was to formulate and elaborate a calcium-fortified bread, optimizing nutritional quality using protein supplementation with regionally produced ingredients that are underutilized for domestic consumption. This will provide nutrients for the prevention of chronic and/or deficiency diseases in the populations of Tucumán and Jujuy.

2. Materials and Methods

2.1. Assessment and Selection of the Theoretical Protein Quality of Bread Formulations

The variability of the protein quality of wheat flour and its mixtures with legume flour was studied. Protein quality was quantified based on the amount and profile of essential amino acids (IAAs), as well as the actual ileal digestibility of protein IAAs using the "Digestible indispensable Amino Acid Score" (DIAAS). The value for each IAA in the diet was calculated, and the lowest value was designated as the DIAAS. For this, the computer tool MixProtLUNA.1-2013 created by the working group [6] was used.

2.2. Materials and Elaborated Bread Proximate Analysis

The commercial wheat flour type 000, white bean (*Phaseolus vulgaris*), pitanga (*Eugenia uniflora* L.), and dry instant yeast Calsa® (Buenos Aires, Argentina) were purchased at a local market in Tucumán, Argentina. The integral flour of white beans was made by grinding and sieving them in a No. 35 sieve. For the elaboration of bread, wheat flour was partially replaced with legume flour (white bean flour) and the mixture of calcium and sodium salts was incorporated, which was obtained in previous works as the optimal mixture for obtaining a dough for bread [7]. The amount of pitanga (*Eugenia uniflora* L.) pulp to be added was studied, which did not modify the textural characteristics of the baked product. The salt substitute mix was made with NaCl and $CaCO_3$ from (Cicarelli® Santa Fé, Argentina). The bread making was carried out in an Atma HP4031E Bread Oven. The elaborated breads were kept in plastic bags of 100 g each in a dry place at room temperature. Mass yield, product yield, and specific volume calculations were performed. The breads were analysed for protein, fibre, fat, ash, and moisture using AOAC [8]. The available carbohydrate content (g/100 g) was calculated by subtracting the contents of moisture, fat, dietary fibre, ash, and protein from 100%. Calcium and sodium content were determined using an atomic absorption spectrometer Perkin Elmer PinAAcle 900T (Akron, OH, USA).

2.3. Determination of Texture and Colour

The textural characteristics of the breads were determined using the TA.XT Plus Texturometer (Stable Micro Systems Ltd., Surrey, UK) containing a 50 kg maximum load cell. The samples were analysed after 10 h of cooking. An average of three measurements was made.

Colour analyses were performed with a Colour Quest XE spectrophotometer (Hunter Lab, Reston, VA, USA) with D65 illuminate, a 0° standard observer, and a 2.5 cm port/viewing area. The determinations were made through the crumb and the upper and lower crusts of the breads ($n = 6$) and was expressed with the L*, a*, and b* parameters.

2.4. Statistical Analysis

Analyses were performed using the IBM SPSS Advanced Statistics 23.0 (IBM Software Group, Chicago, IL, USA). The significant difference between the means was evaluated by Tukey's test ($p < 0.05$) using analysis of variance (ANOVA). All determinations were made in triplicate using different batches of bread samples.

3. Results and Discussion

3.1. Theoretical Protein Quality Assessment

The protein content of the commercial wheat flour used was 10 g/100 g and obtained a DIAAS of 47%, while white bean flour with a protein content of 27.3 g/100 g obtained a DIAAS of 65%. Therefore, the formulation of mixtures of cereals and legumes improves the amino acid balance and translates into a higher value in the quality of the protein compared to that of each one separately. Theoretical combinations were made from additions of 10% to 90% until finding the most suitable proteins for each flour component of the mixture and achieving a product of greater nutritional value using the MixProtLUNA computer program. The results are shown in Table 1 where it is observed that the DIAASs range from 47 to 89%. Theoretical recipes were formulated with the different percentages and then made into breads, each of them with a flour/water ratio of 100/65 and with a content of 1% NaCl salt, 1% calcium salt, and 1% yeast. The bread that finally resulted with adequate baking characteristics was the one that had a 20/80 combination of bean proteins with commercial wheat flour DIAAS: 62.

Table 1. Digestible indispensable amino acid score (DIAAS) in different blends in percentage of wheat flour protein with white bean flour protein.

FOOD/VARIETY/ORIGIN/TYPE White bean flour (*P. Vulgaris*) P: 27.3, D: 65; AALim: SAA Wheat flour (*Triticum* spp.) P:10, D:47; AALim: Lys										
HPB/HT RATIOS										
0–100	10–90	20–80	30–70	40–60	50–50	60–40	70–30	80–20	90–10	100–0
DIAAS %										
47	55	62	69	75	80	84	88	89	79	65

HPB = White bean flour; HT= Wheat flour; DIAAS = Digestible Indispensable Amino Acid Score. AALim: limiting amino acid. Lys: lysine; SAA: sulfur amino acids (Methionine + cysteine) P = Protein g/100 g food.

3.2. Materials and Elaborate Bread Proximate Analysis

Bread is widely consumed; therefore, it could be used as a suitable vehicle to supply high-quality nutrients to consumers without changing their eating habits. A good dough yield of 179% and a bread yield of 90% were obtained (Figure 1). Table 2 shows the chemical composition of the elaborated breads. It can be seen that there were significant differences in all the parameters analysed except for the ash content. Moisture varied from 40.7 to 43.9, being higher in breads with additions of bean flour, pitanga pulp, and calcium salts. Regarding the content of protein, lipids, and dietary fibre, a higher value was obtained in the breads with the addition of bean flour. Regarding the calcium content, it was observed that the content of calcium salts increased in fortified breads with the addition of white bean flour, as expected. Calcium is a nutrient that has limited food sources. Dairy products (milks, yogurts, and cheeses) are the main source of dietary calcium. Despite Argentina being placed among the countries with an important dairy industry, the food availability of calcium is insufficient to cover the population requirements [9]. A serving size of about 40 g of fortified bread already covers 20% of the IDR for an adult (average body weight 70 kg with moderate physical activity, taking into account that the recommendation for calcium intake per day is 1000 mg). The substitution has an inverse effect with respect to sodium intake. Sodium was higher in breads made only with white bean flour, despite this; all baked goods are in accordance with Law 26905 of the Honourable Congress of the Argentine nation's maximum sodium values published in the official bulletin of 2013 number: 32786. Other authors who produced food to cover calcium deficiencies showed similar values, although many of them did not show a sodium decrease and, in some cases, they added sugars. In the future, it would be advisable to carry out acceptability surveys in especially vulnerable groups and populations in which calcium consumption is deficient, as would be case of pregnant and hypertensive people.

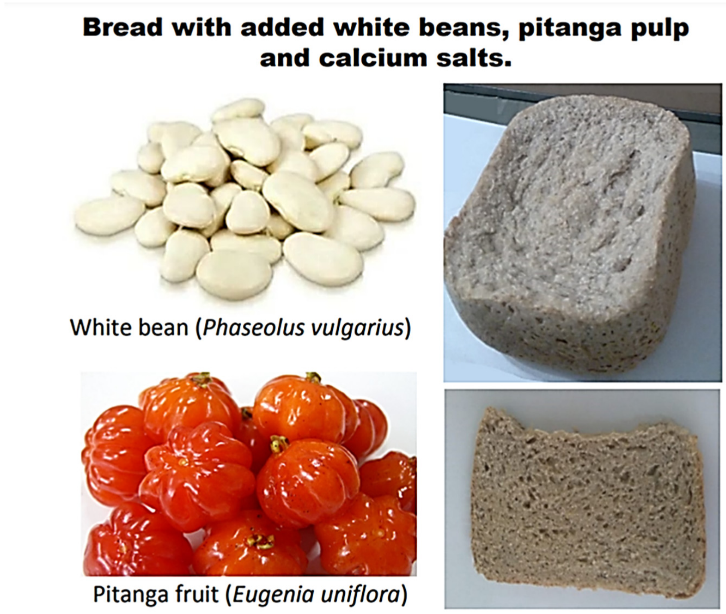

Figure 1. Appearance of pitanga fruit, white beans, and elaborated bread.

Table 2. Proximal compositions (g/100g fresh weight) of different types elaborated breads.

	White Bread	White Calcium Bread **	White Bean Bread	White Bean Calcium Bread **	White Bean Pitanga Bread	White Bean Calcium Pitanga **	*p* Value
Energy (kcal)	220 ± 2 ^{a,b}	221 ± 3 ^{a,b}	229 ± 3 ^{b}	223 ± 1 ^{a,b}	225 ± 5 ^{a,b}	216 ± 4 ^{a}	0.026
Moisture (g)	41.0 ± 0.6 ^{a,b}	40.7 ± 0.7 ^{a}	40.8 ± 0.4 ^{a}	42.5 ± 0.4 ^{b,c}	41.5 ± 0.5 ^{a,b}	42,1 ± 0.7 ^{c}	0.001
CHa (g)	43.4 ^{b}	43.5 ^{d}	35.2 ^{a}	34.4 ^{a}	35.8 ^{a}	33.5 ^{a}	0.000
Protein (g)	9.8 ± 0.0 ^{a}	10.2 ± 0.3 ^{b}	11.3 ± 0.3 ^{b,c}	11.2 ± 0.4 ^{b,c}	11.6 ± 0.6 ^{c}	11.4 ± 0.3 ^{b,c}	0.004
Lipid (g)	0.8 ± 0.2 ^{a}	0.7 ± 0.1 ^{a}	2.7 ± 0.5 ^{b}	2.5 ± 0.7.^{b}	2.0 ± 0.4 ^{b}	2,72 +/- 0,13 ^{b}	0.000
Ash (g)	2.4 ± 0.2	2.0 0.1	2.0 ± 0.4	1.8 ± 0.2	2.0 ± 0.2	2.2 ± 0.3	0.274
DF (g)	2,6 ± 0.5 ^{a}	3,0 ± 0.3 ^{a}	5,9 ± 0.7 ^{b}	5,6 ± 0.4 ^{b,}	7,2 ± 0.1 ^{c}	7,34 ± 0.3 ^{c}	0.000
Ca (mg) #	25.7 ± 0.3 ^{a} (1%)	435.0 ± 25.1 ^{b} (17%)	29.2 ± 3.0 ^{a} (1%)	488.3 ± 22.6 ^{b} (20%)	34.5 ± 3.4 ^{a} (1%)	439.3 ± 38.1 ^{b} (18%)	0.000
Na (mg)	349.5 ± 14.8 ^{a}	331.4 ± 16.4 ^{a}	864.1 ± 11.1 ^{b}	326.7 ± 18.7 ^{a}	835.8 ± 16.1 ^{b}	346.8 ± 18.4 ^{a}	0.000

Mean ± SD; $n = 3$. CHa Carbohydrates available calculated by difference (100-Moisture-Protein-Lipids-Dietary fibre-Ashes). DF: dietary fibre; Ca: calcium (**); Added Ca salts: 2.5 g $CaCO_3$ + 2.5 g $CaCl_2$/500 g of flour. (%) Percentage of coverage of daily intake per 40 g portion of bread # Reference values: IDR: 1000 mg/d according to Ross et al, [10] Different letter in the same line indicated significant difference $p < 0.05$ (Tukey's test).

3.3. Determination of Specific Volume, Texture, and Colour in Elaborated Breads

Table 3 shows the colour measured in the breads made. The results showed significant differences in the four parameters evaluated. The addition of calcium salts increased hardness, produced lighter crumbs and crusts, and did not affect volume with respect to the common white bread. The addition of fruit pulp did modify the colour and specific volume (2.1 mL/g) of the loaves (Table 4). The evaluation of the texture parameters of the breads with legumes, pitanga pulp, and calcium showed significant differences in hardness and chewiness in comparison to the control bread (without added salts or beans), obtaining softer and smoother crumbs in unfortified breads. With the development of these fortified breads, we intended to achieve the revaluation of bean varieties and regional fruits, highlighting their nutritional importance and the agro-industrial potential that they present according to their genetic material. Due to its wide consumption, bread can be used as a suitable vehicle to supply high-quality nutrients to consumers. Therefore, the challenge was to develop a healthy bread that incorporates the consumption of these underutilized products into the daily life of the population. The future research will focus particularly on the acceptability of the bread with white bean flour, calcium, and fruit added.

Table 3. Colour of the crusts and crumbs of breads made with mixtures of wheat flour, beans, and pitanga pulp with and without added calcium salts.

		White Bread	White Calcium Bread	White Bean Bread	White Bean Calcium Bread	White Bean Pitanga Bread	White Bean Calcium Pitanga Bread	*p* Value
Upper crust	L*	74.8 ± 0.5 ^{d}	72.92 ± 0.9 ^{d}	65.97 ± 1.10 ^{b,c}	61.12 ± 1.37 ^{a}	63.99 ± 2.08 ^{b}	66.21 ± 0.92 ^{c}	0.000
	a*	4.1 ± 0.5 ^{a}	4.17 ± 0.39 ^{a}	6.76 ± 0.70 ^{b}	11.63 ± 1.04 ^{c}	6.97 ± 2.89 ^{b}	4.22 ± 0.25 ^{a}	0.000
	b*	24.4 ± 0.7 ^{b}	23.39 ± 0.80 ^{b}	28.76 ± 1.06 ^{c}	36.28 ± 0.66 ^{d}	23.91 ± 1.07 ^{b}	20.24 ± 1.00 ^{a}	0.000
Crumb	L*	65.8 ± 2.3 ^{b}	65.98 ± 0.89 ^{b}	70.36 ± 0.82 ^{c}	68.92 ± 0.90 ^{b,c}	59.07 ± 1.85 ^{a}	61.1 ± 0.6 ^{a}	0.000
	a*	1.8 ± 0.1 ^{a,b}	1.74 ± 0.19 ^{a}	2.61 ± 0.16 ^{c}	3.03 ± 0.12 ^{d}	5.48 ± 0.19 ^{b}	4.7 ± 0.5 ^{a,b}	0.000
	b*	22.7 ± 0.6 ^{c,d}	22.02 ± 0.24 ^{c}	22.98 ± 0.64 ^{d}	24.20 ± 0.28 ^{e}	19.53 ± 0.50 ^{b}	18.1 ± 0.6 ^{a}	0.000
Lower crust	L*	57.1 ± 1.9 ^{c}	62.9 ± 0.7 ^{d}	50.9 ± 1.7 ^{b}	41.1 ± 2.5 ^{a}	55.5 ± 1.5 ^{c}	60.9 ± 0.3 ^{d}	0.000
	a*	13.2 ± 0.7 ^{b}	10.0 ± 0.5 ^{a}	15.1 ± 0.6 ^{c}	18.9 ± 0.7 ^{d}	12.6 ± 0.3 ^{b}	10.7 ± 0.5 ^{a}	0.000
	b*	34.3 ± 0.8 ^{b}	32.8 ± 12 ^{b}	38.7 ± 1.2 ^{c}	45.3 ± 1.9 ^{d}	27.2 ± 1.2 ^{d}	26.4 ± 0.3 ^{a}	0.000

Data shown are mean values of triplicate analyses ± standard deviation from each type of bread. L*, a*, and b* parameters of colour. Values with different letter in the same line indicated significant difference $p < 0.05$ (Tukey's test).

Table 4. Volume and textural profile of breads made with mixtures of wheat flour, beans, and pitanga pulp with and without added calcium salts.

	White Bread	White Calcium Bread	White Bean Bread	White Bean Calcium Bread	White Bean Pitanga Bread	White Bean Calcium Pitanga Bread	*p* Value
Volume (mL/g)	2.8 ^{b}	2.8 ^{b}	2.5 ^{a,b}	2.5 ^{a,b}	2.1 ^{a}	2.0 ^{a}	0.005
Hardness (g)	1208.5 ^{a}	1578.0 ^{b}	1995.3 ^{c}	2807.4 ^{e}	1026.1 ^{a}	2374.9 ^{d}	0.000
Adhesiveness	−0.118	−0.147	−0.265	−0.208	0.000	−0.151	
Springiness	0.914	0.885	0.849	0.843	0.943	0.943	
Cohesiveness	0.813	0.782	0.802	0.750	0.819	0.836	
Gumminess	980.6 ^{a,b}	1229.7 ^{b}	1597.3 ^{c}	2108.2 ^{d}	840.9 ^{a}	1985.6 ^{d}	0.000
Chewiness	896.8 ^{a}	1088.1 ^{a,b}	1355.8 ^{a,b,c}	1781.3 ^{d}	1219.4 ^{a,b,c}	1537.1 ^{c,d}	0.001
Resilience	0.492	0.468	0.469	0.430	0.513	0.536	

Data shown are mean values of triplicate analyses from each type of bread. Values with different letter in the same line are indicated significant difference $p < 0.05$ (Tukey's test).

4. Conclusions

The breads elaborated in this study are inexpensive to produce, made from low-cost crops of regional importance, good quality, and higher contents of protein and calcium. A 40 g serving provides 18% of the RDI for calcium, and due to its ingredients and its nutritional and textural characteristics, it is suitable for inclusion in the diets of vulnerable groups. They will also contribute to the prevention of chronic and/or deficiency diseases. In addition, the use of raw materials of regional origin will encourage local production and therefore support the local economy.

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References

1. Food and Agriculture Organization of the United Nations (FAO); The International Fund for Agricultural Development (IFAD); The United Nations Children's Fund (UNICEF); The World Food Programme (WFP); The World Health Organization (WHO). The State of Food Security and Nutrition in the World 2019: Safeguarding against Economic Slowdowns and Downturns. Rome, Italy, 2019; p. 572. Available online: http://www.fao.org/3/CA3129EN/CA3129EN.pdf (accessed on 15 July 2021).
2. Bassett, M.N.; Gimenez, M.A.; Romaguera, D.; Sammán, N. Nutritional status and food intake of populations from high altitude regions of the Northwest of Argentina. *Arch. Latinoam. Nutr.* **2013**, *63*, 114–124. [PubMed]
3. ENNyS2. Encuesta Nacional de Nutrición y Salud. Informe técnico Septiembre 2019. Ministerio de Salud. Buenos Aires; Argentina. 2019. Available online: http://www.msal.gob.ar/images/stories/bes/graficos/0000001565cnt-ennys2_resumen-ejecutivo-2019.pdf (accessed on 15 July 2021).
4. Bonvecchio, A.; Bernal, J.; Herrera Cuenca, M.; Aldana, M.F.; Gutiérrez, M.; Irizarry, L.; Lay Mendivil, L.; López Bautista, F.; López Reyes, M.; Mata, C.; et al. Recomendaciones de micronutrientes para grupos vulnerables en contexto de desnutrición, durante la pandemia de COVID-19 en Latinoamérica. *Arch. Latinoam. Nutr.* **2019**, *69*, 259–273. [CrossRef]
5. Franzon, R.C.; Carpenedo, S.; Viñoly, M.D.; Raseira, M.d.C.B. Pitanga—*Eugenia uniflora* L. In *Exotic Fruits*; Rodrigues, S., de Oliveira Silva, E., de Brito, E.S., Eds.; Academic Press: Cambridge, MA, USA, 2018; pp. 333–338. [CrossRef]
6. Bassett, M.N.; Espejo, L.M.; Sammán, N.C. Estudio de la calidad de diferentes mezclas de harinas proteicas aplicando el software MixProtLUNA. In Proceedings of the V Congreso Internacional de Ciencia y Tecnología de los Alimentos, Córdoba, Argentina, 17–19 November 2014; pp. 3–10. (In Spanish).
7. Bassett, M.N.; Pérez-Palacios, T.; Cipriano, I.; Cardoso, P.; Ferreira, I.M.; Samman, N.; Pinho, O. Development of bread with NaCl reduction and calcium fortification: Study of its quality characteristics. *J. Food Qual.* **2014**, *37*, 107–116. [CrossRef]
8. AOAC. Association of Official Analytical Chemists. Methods of Analysis (AOAC). Available online: http://www.aoac.org/ (accessed on 1 March 2021).
9. Aballay, L.R.; Eynard, A.R.; Díaz, M.d.P.; Navarro, A.; Muñoz, S.E. Overweight and obesity: A review of their relationship to metabolic syndrome, cardiovascular disease, and cancer in South America. *Nutr. Rev.* **2013**, *71*, 168–179. [CrossRef] [PubMed]
10. Ross, A.C.; Manson, J.E.; Abrams, S.A.; Aloia, J.F.; Brannon, P.M.; Clinton, S.K.; Durazo-Arvizu, R.A.; Gallagher, J.C.; Gallo, R.L.; Jones, G.; et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: What clinicians need to know. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 53–58. [CrossRef] [PubMed]